EGFR Molecular Profiling in Advanced NSCLC
A Prospective Phase II Study in Molecularly/Clinically Selected Patients Pretreated with Chemotherapy

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Introduction: The optimal use of epidermal growth factor receptor (EGFR)-related molecular markers to prospectively identify tyrosine kinase inhibitor (TKI)-sensitive patients, particularly after a previous chemotherapy treatment, is currently under debate.

Methods: We designed a prospective phase II study to evaluate the activity of EGFR-TKI in four different patient groups, according to the combination of molecular (EGFR gene mutations, EGFR gene copy number and protein expression, and phosphorylated AKT expression, pAKT) and clinicopathological (histology and smoking habits) factors. Correlations between molecular alterations and clinical outcome were also explored retrospectively for first-line chemotherapy and EGFR-TKI treatment.

Results: Patients who had progressed during or after first-line chemotherapy were prospectively assigned to EGFR-TKI treatment as follows: (G1) EGFR mutation (n = 12); (G2) highly polysomic/amplified EGFR (n = 18); (G3) EGFR and/or pAKT positive (n = 41); (G4) adenocarcinoma/bronchoalveolar carcinoma and no smoking history (n = 15). G1 and G4 had the best and second-best overall response rate (25% and 20%, respectively), whereas the worst outcome was observed in G2 (ORR, 6%; p = 0.05). Disease control was highest in G1 and G4 (>50%) and lowest in G3 (<20%) (p = 0.02). Patients selected by EGFR mutation or clinical parameters (G1 and G4) also had significantly better progression-free survival and overall survival (p = 0.02 and p = 0.01, respectively). Multivariate analysis confirmed the impact of sex, smoking history, EGFR/KRAS mutation, and pAKT on outcomes and allowed us to derive an efficient predictive model. Histology, EGFR mutations, and pAKT were independent predictors of response to first-line chemotherapy at retrospective analysis, whereas pAKT and human epidermal growth factor receptor 2 expression were the only independent predictors of progression-free survival and overall survival.

Conclusions: Selection of patients based on either EGFR mutation or clinical characteristics seems an effective approach to optimize EGFR-TKI treatment in chemotherapy-pretreated non–small-cell lung cancer patients.

Key Words: Non–small-cell lung cancer, EGFR, KRAS, Tyrosine kinase inhibitors.

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Lung cancer is the leading cause of cancer deaths worldwide, regardless of sex, and has an overall 5-year survival rate of approximately 15%.1,2 Non–small-cell lung cancer (NSCLC) accounts for about 80% of all lung cancers3 and its dismal prognosis is heavily influenced by the fact that the majority of patients presents with advanced, inoperable disease at diagnosis.4,5 Although palliative chemotherapy has led to minimal progress in the past 10 years,4 we have recently witnessed a major revolution in the approach to the systemic treatment of advanced disease. Indeed, the introduction of small-molecule inhibitors of the tyrosine kinase activity of the epidermal growth factor receptor (EGFR-TKI)4,6 and the discovery of somatic, activating EGFR mutations in lung adenocarcinoma7,8 have opened new scenarios in the management of inoperable NSCLC.9,10

The role of the EGFR-TKI as the best first-line therapeutic option for patients with advanced NSCLC harboring a mutated EGFR (EGFR-M+) has been firmly established by the retrospective analysis of EGFR-M+ patients included in two large phase III trials conducted in Asian patients with lung adenocarcinoma9,11 and by three prospective randomized trials selectively accruing EGFR-M+ patients.12,13 A recent meta-analysis of these five trials conducted by our group, encompassing 805 Asian patients with EGFR-M+,16 unequivocally demonstrates EGFR-TKI superiority (approximately 25%...
increase in progression-free survival [PFS] and overall response rate [ORR]), as compared to standard, platinum-based first-line chemotherapy, with significantly less toxicity. Conversely, patient selection for second and subsequent lines of treatment with EGFR-TKI using EGFR molecular profiling (including mutational analysis) is more controversial. Among other factors, such controversy stems from the lack of evidence of a clear benefit from erlotinib treatment in EGFR-M+ included in the retrospective analysis of the BR.21 trial and from the suggestion that other subgroups of NSCLC (patients with EGFR gene amplification, EGFR overexpression by immunohistochemistry [IHC], constitutive AKT phosphorylation, etc.) may also derive substantial benefit from EGFR-TKI treatment. However, these data are mostly derived from small-scale, often retrospective, single-arm studies that employed individual selection markers, rather than comprehensive assessments of the EGFR molecular profile. As a result, the use of molecular selection markers other than EGFR mutation testing for EGFR-TKI treatment assignment remains speculative at present, particularly for patients who have already undergone first-line chemotherapy for advanced disease.

We therefore set out to prospectively investigate the activity of EGFR-TKI as second or subsequent line treatment in molecularly defined subgroups of patients with advanced NSCLC, using three commonly used selection parameters (EGFR mutation, EGFR gene amplification/high-grade polysomy, EGFR and/or pAKT overexpression). An additional group of patients, in whom molecular tests were negative or could not be performed, was selected on the basis of purely clinical factors (adenocarcinoma histology and no smoking history), taking into account the significant fraction of patients for whom adequate tissue is not obtainable in routine clinical practice. Finally, the impact of EGFR molecular profiling on the outcome after standard first-line chemotherapy was also analyzed retrospectively.

**PATIENTS AND METHODS**

**Study Design**

The study was designed as a prospective phase II study evaluating EGFR-TKI (gefitinib—Iressa, Astrazeneca Inc., London, UK, or erlotinib—Tarceva, Hoffman-LaRoche, Basel, Switzerland) as a second or subsequent line of treatment in advanced NSCLC patients progressing after at least one line of standard chemotherapy. Patients were assigned to treatment according to four different clinical/pathological groups: group 1 (G1), mutated EGFR; group 2 (G2), EGFR mutation negative or unknown, amplified EGFR gene, and/or high-grade chromosome no. 7 polysomy; group 3 (G3), EGFR mutation/amplification negative/unknown, positive EGFR and/or pAKT IHC staining; group 4 (G4), molecular profile negative for parameters defining groups 1 to 3 or not evaluable, adenocarcinoma or bronchoalveolar carcinoma histology, and no smoking history (never smokers: <100 cigarettes in lifetime; former smokers: smoking cessation ≥6 months before starting therapy). Main inclusion criteria encompassed: histologically or cytologically proven stage-IIIIB or -IV NSCLC; at least one previous line of cytotoxic chemotherapy for advanced disease (TKI were allowed as first-line treatment for advanced disease for patients who progressed during or shortly [<6 months] after platinum-based neoadjuvant/adjuvant chemotherapy); Eastern Cooperative Oncology Group performance status of 0 to 2; measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST) criteria; other standard eligibility criteria for EGFR-TKI treatment also applied. The study was approved by the local ethics committee. Eligible and consenting patients received gefitinib 250 mg/d or erlotinib 150 mg/d until disease progression, unacceptable toxicity, or consent withdrawal.

**Sample Size Calculation and Statistical Analysis**

Objective response was the primary end point of the study; using a single-stage design as described by A’Hern, a sample size of 40 patients was considered sufficient to yield an 80% probability (1 – β) of rejecting a baseline response rate of 10% (p0) when the true response rate was 25% (p1), with an exact 5% one-sided significance test (α). The study was to be stopped and EGFR-TKI treatment rejected for that specific group if less than eight objective responses were observed in any of the clinical/pathological groups. Descriptive statistics were used to summarize pertinent study information. Optimal cutoff values for pAkt and EGFR expression levels as detected by IHC were selected using maximally selected log-rank statistics (Supplemental Figure S1, Supplemental Digital Content 1, http://links.lww.com/JTO/A248). The association among genetic variables, biopathologic characteristics, and response/disease control rate (DCR) was tested by Pearson’s χ² or Fisher’s exact test, as appropriate. Multiple correspondence analysis (MCA), a descriptive/exploratory technique designed to analyze simple two-way and multiway tables, was used to identify the association of multiple variables into complex biological profiles. ORR, according to the RECIST criteria, and DCR (defined as the combination of patients achieving an objective response [OR] or disease stabilization for ≥6 months) were derived with 95% confidence intervals (CIs). Univariate and multivariate logistic regression analyses were used to assess the impact of different variables on DCR, after adjusting for the effect of all other variables. Results are reported as OR, with 95% CI. Overall survival (OS), defined as the time between treatment start and death for any cause) and PFS (defined as the time between treatment start and progression or death for any cause) were calculated by the Kaplan-Meier product-limit method. The log-rank test was used to assess differences between subgroups. Significance was defined at the p < 0.05 level. Hazard ratios and 95% CI were estimated for each variable using the Cox univariate model. A multivariate Cox proportional hazard model was also developed using stepwise regression (forward selection); enter- and remove-limits for logistic and Cox multivariate analysis were p = 0.10 and p = 0.15, respectively. A logistic equation including the coefficients of the regression analysis was then constructed to calculate an estimation of individual patient’s probability of PFS and OS upon EGFR-TKI treatment: probability of 6-month PFS/1-year OS = (Exp[(X × B1 + intercept)])1 + (Exp[(X × B1 + intercept)])A, where X × B is the coefficient B for each single confounding factor X.24,25 The SPSS (version
EGFR Molecular Profiling

Genomic DNA was isolated by standard procedures. Genetic analysis of the EGFR gene was carried out as previously described, for the detection of KRAS mutations a recently described mutation-enriched sequencing method was used. EGFR fluorescence in-situ hybridization assay was carried out using the locus-specific identifier EGFR (Spectrum Orange) and the chromosome enumeration probe 7 (Spectrum Green) probes (Vysis, Downers Grove, IL), as previously described. Fluorochrome signals were captured individually and images were generated using the Quips Genetic Workstations and Imaging Software (Vysis). Slides were analyzed at 1000 magnification. At least 100 well-defined nuclei were scored for each hybridization. Amplification was defined as an EGFR to CEP7 ratio greater than two. Polysomy levels were stratified into low- and high-grade according to a recently proposed scoring system. EGFR expression was assessed by indirect immunoperoxidase staining using EGFR-pharmDx-kit (Dako, Milan, Italy). Human epidermal growth factor receptor 2 (HER-2) and phospho-AKT expression were assessed by indirect immunoperoxidase staining after pretreatment of the sections in a thermostatic bath at 96°C for 40 minutes in 10-mM citrate buffer (pH 6). Sections were then incubated with an anti-HER-2 polyclonal antibody (A0485, Dako) or with two polyclonal antibodies against phosphorylated AKT (Ser473, pAKT). Immunostaining was revealed by a streptavidin–biotin–enhanced immunoperoxidase technique (SuperSensitive MultiLink, Novocastra, Menarini, Florence, Italy) in an automated autostainer (Bond Max, Menarini). EGFR, HER-2, and pAKT expression were scored considering both staining intensity (0, 1+ , 2+ , and 3+) and percentage of positive cells. A hybrid variable was then created by multiplying staining intensity by the percentage of positive cells (0–100), as recently suggested. For HER-2 protein expression, we used the Herceptest scoring system and regarded 0 to 1+ cases as negative and 2+ to 3+ cases as positive.

RESULTS

Study Population and Molecular Analysis

From March 2005 to December 2007, 188 patients referred to our Institution for advanced (stage IIIIB or IV), pretreated NSCLCs were screened for molecular alterations along the EGFR pathway (Fig. 1). Clinical and biological characteristics are listed in Supplemental Table S1 (Supplemental Digital Content 2, http://links.lww.com/JTO/A249). One or more of the planned molecular analyses could not be performed in a fraction of patients, ranging from 24 (not evaluable for EGFR amplification) to 40% or more (not evaluable for EGFR and KRAS mutations), because of the lack of adequate tissue sampling (inadequate fixation, small biopsies, inadequate tumor sampling, and cytology only) or the inability to obtain paraffin-embedded tumor blocks for patients referred from different institutions. First, we explored the relationships between clinical/pathological and molecular parameters using MCA.

In addition to the expected association among female sex, smoking history, adenocarcinoma histology, and EGFR mutations, MCA revealed a close association among EGFR, pAKT, and HER-2 expression, and KRAS mutations (Supplemental Figure S2, Supplemental Digital Content 3, http://links.lww.com/JTO/A250); logistic regression analysis confirmed statistically significant associations between selected clinical and biological parameters (Supplemental Table S2, Supplemental Digital Content 4, http://links.lww.com/JTO/A251).

EGFR Pathway Status and Outcome after EGFR-TKI Treatment

Upon progression after previous chemotherapy (neoadjuvant/adjuvant: n = 14; first line: n = 51; second line or subsequent lines: n = 21), 86 patients were prospectively assigned to EGFR-TKI treatment (gefitinib: n = 41; erlotinib: n = 45) according to one of the following clinical/molecular groups: (G1), mutated EGFR (n = 12); (G2), EGFR mutation negative/unknown, amplified EGFR gene and/or high-grade chromosome no. 7 polysomy (n = 18); (G3), EGFR mutation/amplification negative/unknown, positive EGFR and/or pAKT IHC staining (n = 41); and (G4), molecular profile negative or not evaluable for parameters defining groups 1 to 3, adenocarcinoma or bronchoalveolar carcinoma histology and no smoking history (n = 15) (Fig. 1). The presence of KRAS mutations was also analyzed in all patients with adequate material, but was not used as a selection criterion. ORR and DCR in the entire cohort were 12% (95% CI, 5–18%) and 34% (95% CI, 24–44%), respectively. Complete and partial responses occurred more frequently in G1 (ORR, 25%) and G4 (ORR, 20%), whereas G2 and G3 had a statistically significant worse outcome in terms of OR (p = 0.05). Similarly, DCR was highest in EGFR-mutated patients (G1: 58%) and progressively decreased in clinically selected (G4: 53%), EGFR-amplified/polysomic (G2: 33%), and EGFR/pAKT overexpressing patients (G3: 19%) (p = 0.02, Fig. 2A and B). Median PFS and OS in the entire cohort were
4 months (95% CI, 3–5 months) and 9 months (95% CI, 5–12 months), respectively. When PFS and OS outcomes were analyzed according to molecular/clinical group classification, significant differences were found ($p = 0.02$ and $p = 0.01$, respectively): indeed, patients in G1 and G4 had longer PFS (median: 7 and 10 months, respectively), as compared to patients in G2 and G3 (median PFS: 5 and 4 months, respectively); OS was similar in G1, G2, and G4 (median OS: 24, 18, and 18 months, respectively) and shorter in G3 (median OS: 6 months) (Fig. 2C and D). Toxicity was within the expected range for pretreated NSCLC patients undergoing EGFR-TKI treatment, both qualitatively and quantitatively, with 8% grade 3 to 4 skin toxicity, 3% grade 3 to 4 diarrhea, and 5% of patients who required temporary treatment interruptions and/or dose reductions (data not shown). To further confirm these results, we retrospectively analyzed the entire cohort of screened patients treated with EGFR-TKI, which included 13 additional patients who did not belong to any of the abovementioned prospective groups ($n = 99$). Such a retrospective analysis served two main purposes: first, the choice of prospective selection factors was made a priori and did not allow checking for the influence of factors that could not be accounted for in the design of the prospective groups; second, multivariate analysis performed on the entire cohort of treated patients was also instrumental in the construction of a model for the prediction of individual patient probability of outcome (see below).

Low pAKT expression was the only independent predictor of better DCR at multivariate analysis ($p = 0.0005$). No smoking history ($p = 0.006$), EGFR mutations ($p = 0.091$), absence of KRAS mutation ($p = 0.025$), and low pAKT expression ($p < 0.0001$) were independent predictors of longer PFS; similarly, when OS was considered, female sex ($p = 0.005$), EGFR mutations ($p = 0.062$), absence of KRAS mutations ($p < 0.0001$), and low pAKT expression ($p < 0.0001$) were independent predictors of longer OS (Table 1 and Fig. 3). The accuracy of the derived, four-variable, multivariate models was further verified by receiver-operating characteristic analysis, which demonstrated an area under the curve of 0.82 (95% CI, 0.73–0.90) and 0.73 (95% CI, 0.63–0.81) for PFS and OS, respectively (Supplemental Figure S3, Supplemental Digital Content 5, http://links.lww.com/JTO/A252). In addition, such multivariate models were able to predict an individual patient probability of a 6-month PFS ranging from 1.4 to 84.3% (Supplemental Figure S4, Supplemental Digital Content 6, http://links.lww.com/JTO/A253) or an individual patient probability of 1-year OS ranging from 8.8 to 57.5% (Fig. 4).

**EGFR Pathway Status and Outcome after First-Line Chemotherapy**

One hundred forty-four patients were evaluable for clinical outcome following first-line chemotherapy, which consisted of platinum-based doublets in the majority of patients,
TABLE 1. Multivariate Analysis of Factors Influencing DCR, PFS, and OS upon TKI Treatment

<table>
<thead>
<tr>
<th>Disease Control Rate</th>
<th>OR (95% CI)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pAKT (&lt;120 vs. &gt;120)</td>
<td>8.999 (1.972–41.069)</td>
<td>0.005</td>
</tr>
<tr>
<td>Progression-Free Survival</td>
<td>HR (95% CI)</td>
<td>p Value</td>
</tr>
<tr>
<td>Smoking history</td>
<td>NA</td>
<td>0.023</td>
</tr>
<tr>
<td>Current vs. never</td>
<td>2.232 (1.255–3.972)</td>
<td>0.006</td>
</tr>
<tr>
<td>Former vs. never</td>
<td>1.482 (0.862–2.548)</td>
<td>0.155</td>
</tr>
<tr>
<td>Current vs. former</td>
<td>0.664 (0.364–1.212)</td>
<td>0.182</td>
</tr>
<tr>
<td>EGFR mut (no vs. yes)</td>
<td>2.078 (0.889–4.854)</td>
<td>0.091</td>
</tr>
<tr>
<td>KRAS (pos vs. neg)</td>
<td>2.484 (1.120–5.006)</td>
<td>0.025</td>
</tr>
<tr>
<td>pAKT (&lt;120 vs. &gt;120)</td>
<td>3.220 (1.875–5.529)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Overall Survival</td>
<td>HR (95% CI)</td>
<td>p Value</td>
</tr>
<tr>
<td>EGFR mut (no vs. yes)</td>
<td>2.749 (0.949–7.961)</td>
<td>0.062</td>
</tr>
<tr>
<td>pAKT (&lt;120 vs. &gt;120)</td>
<td>4.242 (2.230–8.072)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex (male vs. female)</td>
<td>2.296 (1.280–4.119)</td>
<td>0.005</td>
</tr>
<tr>
<td>KRAS (pos vs. neg)</td>
<td>4.704 (2.074–10.668)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

DCR, disease control rate; PFS, progression-free survival; OS, overall survival; TKI, tyrosine kinase inhibitor; pAKT, phosphorylated AKT; OR, odds ratio; HR, hazard ratio; CI, confidence interval; mut, mutation; pos, positive; neg, negative; EGFR, epidermal growth factor receptor.

and were retrospectively analyzed in relation to clinical/pathological factors and molecular alterations along the EGFR pathway (Fig. 1 and Supplemental Table S1, Supplemental Digital Content 2, http://links.lww.com/JTO/A249). ORR in the entire population was 21% (95% CI, 14–27%); multivariate analysis indicated squamous histology ($p \leq 0.02$), $EGFR$ mutations ($p = 0.061$), and low pAKT expression ($p = 0.056$) as independent predictors of response to first-line treatment (Supplemental Table S3, Supplemental Digital Content 7, http://links.lww.com/JTO/A254). Classification and regression tree analysis confirmed that the first node identifying patients at higher likelihood of response was squamous versus nonsquamous histology (ORR, 43% versus 18%); within the nonsquamous subgroup, $EGFR$-mutated patients had a better response (ORR, 38% versus 16%); finally, in patients without $EGFR$ mutations, treatment response was better in low- versus high-pAKT expressors (ORR, 20% versus 4%, data not shown). Median PFS and OS for the entire population were 6 months (95% CI, 5–8) and 19 months (95% CI, 14–24), respectively (Fig. 5). Multivariate analysis indicated HER-2 and pAKT overexpression as independent predictors of shorter PFS ($p = 0.047$ and $p = 0.006$, respectively); the same factors were also independent predictors of shorter OS ($p = 0.005$ and $p = 0.001$, respectively); in addition, subsequent EGFR-TKI treatment positively impacted on OS ($p < 0.0001$) (Supplemental Table S3, Supplemental Digital Content 7, http://links.lww.com/JTO/A254, and Fig. 5).

DISCUSSION

To the best of our knowledge, this is the first study to prospectively select pretreated NSCLC patients for EGFR-TKI treatment based on the four most widely used clinicopathological predictive factors, i.e., $EGFR$ mutation status,
EGFR amplification/high-grade polysomy, EGFR/pAKT protein overexpression, and adenocarcinoma histology/no smoking history. Based on activity and survival data obtained in the four prospective patient cohorts, patients harboring an EGFR mutation and patients with adenocarcinoma histology and no smoking history (G1 and G4) seem to be at highest likelihood of achieving prolonged disease control and survival. Conversely, molecular profiling of the EGFR pathway does not seem to influence outcome upon standard first-line chemotherapy, with the notable exception of pAKT and HER-2 protein expression, which are powerful negative prognostic factors in the first-line setting. In addition, retrospective analysis of the patient cohort exposed to EGFR-TKI allowed us to derive a prognostic/predictive model that very efficiently discriminated the individual patient probability of being alive and/or progression free on the basis of a relatively simple combination of four clinical/molecular factors. As the use of two different EGFR-TKI (erlotinib and gefitinib) may have influenced the results obtained, we also retrospectively analyzed the potential impact of individual drugs on outcome in the entire cohort of EGFR-TKI–treated patients and found no significant association among the use of erlotinib or gefitinib and DCR, PFS, and OS at univariate analysis, suggesting that the overall outcome was independent of the specific drug used.

Although the impact of different patient selection approaches on the performance of EGFR-TKI in the first-line setting is nowadays relatively clear (i.e., highly significant ORR/PFS benefit for EGFR-TKI in EGFR-M+ patients and highly significant PFS/OS detriment for EGFR-TKI in unselected or EGFR-mutation–negative patients), the second-line setting still represents a clinical and methodological challenge. Indeed, direct comparison of second-line gefitinib to docetaxel in unselected NSCLC populations demonstrates therapeutic equivalence in a recent meta-analysis phase II/III randomized studies and even suggests an ORR/PFS benefit for gefitinib in molecularly unselected patients of Asian origin. In contrast to the first-line setting, no prospective data from randomized trials conducted in selected (either on clinical or molecular grounds) patient populations in second-line are currently available. Most of the proposed clinical/molecular selection factors have been identified by retrospective analyses and, in the best-case scenario, have been evaluated in prospective, nonrandomized phase II trials. Another level of complexity in the interpretation of such data comes from the attrition rate generated by the relatively low sample availability for molecular analysis in retrospective analyses of prospective trials conducted in unselected populations (e.g., only 32% of the patients could be tested for EGFR mutations in the BR.21 study, and from the inconsistency in the choice of the comparator arm [active treatment versus placebo]). Although the patient-grouping scheme employed in our study was relatively complicated, such design was functional to the clear identification of individual clinical/molecular selection factors. Indeed, the lack of a comprehensive analysis of such factors (i.e., the use of single potential predictors or, at the opposite extreme, the use of mixed eligibility criteria) may have contributed to the present uncertainties in the identification of patients who may benefit most from EGFR inhibition; this is best exemplified by the post hoc analysis of EGFR mutations and gene amplification data in the IPASS trial, in which 81% of the patients with high EGFR gene copy number also had EGFR mutations, possibly leading to the conclusion that EGFR gene copy number may drive sensitivity to EGFR-TKI; instead, patients with EGFR mutations had a significantly better PFS with gefitinib irrespective of EGFR gene copy number, whereas nonmutated patients did uniformly better on chemotherapy in both high- and low-EGFR copy number groups.

Although excluding patients with unknown EGFR gene or protein status would have resulted in cleaner and easier-to-interpret results, we elected to retain such patients in our study design to better reflect the “real-world” situation that clinicians often face in their routine practice; indeed, a relatively high percentage of patients (22–40% in the present series) lacks adequate material for one or more molecular analyses. From a practical standpoint, the management of such patients is an important clinical challenge as we have to balance the need to acquire all the necessary molecular information, sometimes repeating or performing de novo invasive and relatively risky procedures, with time constraints and the individual clinical situation that, especially in a chemotherapy-pretreated, advanced NSCLC patient may not warrant an aggressive diagnostic approach. In such complicated cases, data obtained in

**FIGURE 4.** Individual patient probability of 1-year overall survival according to different combinations of clinical/molecular factors found to be independent predictors of outcome upon EGFR-TKI treatment at multivariate analysis (see also Table 1). Accuracy of the multivariate model used to calculate individual patient probability of outcome was assessed by receiver-operating characteristic analysis (area under the curve 0.73; 95% CI, 0.63–0.81, see Supplemental Figure S3, Supplemental Digital Content 5, http://links.lww.com/JTO/A252). EGFR, epidermal growth factor receptor; pAKT, phosphorylated AKT.
relatively “pure” patient populations (typical of clinical trials) are not readily applicable. For the very same reason, although the results of prospective trials conducted in patients selected on the basis of purely clinical factors (histology, smoking history, and ethnicity) have not had a major impact as compared to historical data in unselected patients, we also elected to retain a group of patients selected only on clinicopathological grounds (G4). In such a real-world situation, our data clearly indicate that, when molecular analysis is not available or when a patient is negative for EGFR mutation, amplification, or IHC, the use of an EGFR-TKI is a reasonable option if the patient has never smoked and has adenocarcinoma histology.

FIGURE 5. Kaplan-Meier survival plots for PFS (left column) and OS (right column) after first-line chemotherapy according to clinical/molecular factors found to be independent predictors of outcome at multivariate analysis (Supplemental Table S3, Supplemental Digital Content 7, http://links.lww.com/JTO/A254). Log-rank $p$ values are shown for each individual panel. PFS, progression-free survival; OS, overall survival; HER-2, human epidermal growth factor receptor 2; pAKT, phosphorylated AKT; TKI, tyrosine kinase inhibitor; pos, positive; neg, negative.
In this respect, the outcome of clinically selected patients (G4) is actually superimposable to that of EGFR-M+ patients.

One possible confounding factor in the interpretation of these results is obviously the prognostic impact that the molecular factors adopted for patient selection may have on the natural history of the disease per se, regardless of the treatment employed. This is particularly true for EGFR mutations, which are commonly believed to portend increased sensitivity not only to EGFR-TKI but also to standard chemotherapy. This conclusion is mostly based on the retrospective analysis of the TRIBUTE first-line study, in which EGFR-M+ patients fared significantly better independently of the treatment assigned.35 However, other lines of evidence do not support a “prognostic” value of EGFR mutations: (1) EGFR mutations have no prognostic impact in series of NSCLC patients undergoing surgery (perhaps the best model to appreciate the impact of a molecular factor on the natural history of the disease),38–41; (2) EGFR mutations were not prognostic in the placebo arm of the SATURN maintenance trial;42 (3) although ORR was higher, survival outcomes were not statistically different in EGFR-M+ patients assigned to chemotherapy, as compared to EGFR-wt patients, in the IPASS trial.11 We assessed whether the group assignment might per se have influenced the results of EGFR-TKI treatment, by selecting patients with different prognostic characteristics independent of the treatment. When the group assignment was applied retrospectively to the 144 patients treated with first-line chemotherapy, no statistically significant difference was found for PFS across all groups (Supplemental Figure S5, Supplemental Digital Content 8, http://links.lww.com/JTO/A255), thus suggesting the absence of a confounding “prognostic” effect.

Although KRAS mutations were identified in NSCLC tumors more than 20 years ago, we have only just begun to appreciate the clinical value of KRAS tumor status. The occurrence of KRAS mutations has been linked to resistance to small-molecule EGFR-TKI,27,43 whereas its relationship to the outcome upon treatment with anti-EGFR mAbs seems to differ depending on the context in which it is analyzed (colorectal cancer versus NSCLC). Given the lack of a consensus, we elected not to use KRAS mutations for patient selection for EGFR-TKI treatment in this study; however, consistent with previous findings,19,27,43 the presence of KRAS mutations was a strong negative predictive factor for PFS and OS at multivariate analysis in our series of patients treated with EGFR-TKI.

Finally, multivariate analysis of the data reported herein allowed to derive a prognostic/predictive model that very efficiently discriminated the individual patient probability of being alive and/or progression free on the basis of a relatively simple combination of five clinical/molecular factors. Other prognostic/predictive models or risk indexes for EGFR-TKI have been proposed,44,45 mostly based on purely clinical factors, such as performance status, serum lactate dehydrogenase and hemoglobin levels, weight loss, response to prior chemotherapy, and occurrence of skin rash upon EGFR-TKI treatment; although the discrimination power of such models was good, they are more likely to reflect tumor burden and growth rate, rather than underlying biology, thus being potentially applicable to any kind of second-line or subsequent line treatment. Although our individual patient probability model obviously requires validation in larger and independent series, it does have several interesting features: (1) the only two clinical variables that entered the final model (sex and smoking habit) are actually independent predictors of higher ORR and prolonged PFS even in the selected EGFR-M+ population, as demonstrated by our recent meta-analysis46; (2) there are categories of patients with wt-EGFR that have an individual patient probability of survival upon EGFR-TKI treatment that is similar or higher to that of EGFR-M+ patients (e.g., females with KRAS mutations, wt-EGFR, and low pAKT) have the slightly higher probability of 1-year survival as compared with males with wt-KRAS, EGFR-M+, and high pAKT (Fig. 4 and Supplemental Figure 4, Supplemental Digital Content 6, http://links.lww.com/JTO/A253), which raises interesting hypotheses for further biological, and possibly clinical, studies.

In summary, with all the limitations of uncontrolled, single-arm, phase II trials, our study in a real-world population of patients does indicate that selection of patients for EGFR-TKI treatment upon progression after first-line chemotherapy should be based on EGFR mutation status whenever possible, but it can be also effectively be supported by clinical selection factors (adenocarcinoma histology and no smoking history) when mutational analysis is not available. Moreover, approaches to treatment personalization based on individual patient probability of outcome deserve further clinical investigation and may shed light on the biology underlying sensitivity/resistance to EGFR-TKI.

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